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Overview of the Cell Therapy Field

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1.1 The Context of Cell Therapies and Their Manufacturing Challenges

Cell therapies are not new. The first cell therapy was the transplant of bone marrow stem cells for patients with leukaemia in the late 1970s (Thomas et al., 1975). Over the next 20 years bone marrow stem cell transplants were adapted and adopted for bone marrow cancers and extended into other clinical indications, for example, inherited immunodeficiency. Bone marrow stem cell transplantation is now a routine clinical procedure for multiple indications.

The example of bone marrow stem cell therapies illustrates several of the characteristics that define cellular therapies more broadly. They were developed for less prevalent indications. They are allogeneic (see Box 1.1) one donor-one recipient therapies that were developed wholly by clinicians in a hospital context. They require interventional clinical procedures for administration of the therapy. They led to widespread clinician-led adoption by the clinical community through global clinician networks. They defined an approach to the **safety** of cell therapies based on risk and benefit to patients. They encouraged the development of cell processing expertise within hospitals and in many ways provided a basis of skills and expertise for clinicians to facilitate the development of other cell

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therapies (Foley and Whitaker, 2012). Though led by clinicians, the development and widespread adoption of bone marrow stem cell therapies was facilitated by companies who provided high-value goods and services to help manufacture and deliver the therapies in a hospital context. Around the turn of the millennium, two cell therapy products developed by companies were the first cell-based therapies to be approved by the US Food and Drug Administration (FDA). Apligraf and Dermagraft were competing skin-equivalent products designed to improve the healing of wounds and burns (Kemp, 2006).

These two therapies illustrate some of the characteristics of company-led approaches to cell therapies. They are allogeneic, one donor-many recipient cell therapy products that are manufactured at scale for prevalent indications. They do not require complex clinical procedures (Foley and Whitaker, 2012).

Apligraf and Dermagraft initially failed in the market (Lysaght and Hazlehurst, 2004). One key reason was cost of goods: the products were manufactured manually and had a short shelf life – two aspects

Box 1.1 Cell Therapy Definitions

Autologous

The patient's cells are the cells used in the therapy. No immune response is expected.

Allogeneic

The cell source is different to the patient receiving the cell therapy. There is a possibility of an immune response.

One to one

The cells used in the therapeutic dose are only of sufficient quantity to treat one patient; these treatments can be autologous or allogeneic.

One to many

The cells used are amplified to a scale able to treat many patients; these treatments can only be allogeneic.

of manufacture and distribution that are not well suited to prevalent indications. A contributing factor was cost relative to existing treatments, despite improved efficacy (see Box 1.2).

Box 1.2 Dermagraft and Apligraf – a roller coaster of investment, manufacturing costs and reimbursement

In the 1990s, Advanced Tissue Sciences invested around $300m to develop Dermagraft and Transcyte for the treatment of diabetic foot ulcers. In 2000, ATS formed a marketing partnership with Smith and Nephew, a global leader in wound care products. Dermagraft was approved by the FDA in 2001. In 2002, ATS filed for bankruptcy. In 2003, Smith and Nephew purchased ATS from bankruptcy and continued with manufacturing and sales. Smith and Nephew ceased production in 2005. In 2006, Advanced Biohealing purchased the Smith and Nephew manufacturing assets for an undisclosed amount (Jones, 2011), presumably at a value destroying discount, and in 2007 resumed manufacture, with a sales/reimbursement model that led to $147m sales in 2010. In 2011, Shire bought ABH for $750m (Smith, 2014). In 2013, Dermagraft assets were declared at $683m on Shire's balance sheet and 9 month losses for Dermagraft were $324m (Reporter, 2014).

Organogenesis was the first to receive FDA approval for a living, allogeneic, cell-based product (Apligraf). They were successful in securing a marketing agreement with Novartis in 1996 (Connolly, 2002a). However, the cost of producing Apligraf was too high and in 2002 Organogenesis filed for bankruptcy and terminated its marketing agreement with Novartis. A short-term deal with Novartis and company restructure (Connolly, 2002b) today means that Organogenesis develops, manufactures and markets its own products.

In early 2014, Organogenesis acquired Dermagraft from Shire, with a promise of a $300m payment based on future sales, but without accepting liability for the ongoing Department of Justice investigation into ABH sales and marketing practices (GenEngNews, 2014). Later that year, Medicare altered reimbursement rules (Carroll, 2013), suggesting that the $1,500 cost of Dermagraft would be reimbursed at a maximum of $840. Dermagraft is a very effective treatment for diabetic ulcers, but costs and reimbursement routes may prevent it reaching patients.

1.1.1 Regulation of Cell Therapies

Neither bone marrow stem cell transplantation nor the first two marketed cellular products were regulated as cell therapy products now are. A key question in the current regulatory landscape is whether cells are substantially manipulated before administration to the patient. Minimally manipulated cells, for example, using aseptic separation or enrichment, are governed by the same regulations that apply to any cell or tissue taken from a patient. Therapeutics that involve any more substantial manipulation including expanding cell numbers are now governed by different and more stringent rules akin to those used in the regulation of other medicinal products such as small molecule pharmaceuticals and biologics. These rules require that quality, safety and efficacy are demonstrated to the satisfaction of the regulators, both in order to undertake the clinical trials and for authorization as an approved medicinal product if the trials are successful. The rules include a requirement to show that the product has been manufactured according to Good Manufacturing Practice. So products similar to Apligraf and Dermagraft now require these new authorizations, while bone marrow stem cell transplantation with its minimal manipulation before administration does not and is overseen for Good Clinical Practice by organizations such as JACIE in Europe. In the US, the FDA oversees Good Clinical Practice for bone marrow transplantation, but this is separate from Biologics manufacture; in the US, the term "Biologics" encompasses cell therapies and the more traditional biopharmaceuticals (Oancea et al., 2012).

The uncertainties that preceded the introduction of the new regulations and the costs in time and money that were required for compliance with the new regulations led to a pause in the development of cell therapies, above all in the US and Europe. Nonetheless it is notable that both clinician- and company-led cell therapies have adapted to the regulatory change. As an example of the former, 12 of the 26 cell therapy manufacturing facilities in the UK are now accredited for Good Manufacturing Practice-compliant manufacture of cellular products (Foley et al., 2012). It should be acknowledged that the new regulations for cell therapies are very similar to those for biologics, and so are well understood by the pharmaceutical sector. They do however still pose a substantial manufacturing challenge, since it is the cells themselves, not a biotherapeutic product produced by cells, that are the medicinal product (the ATMP: Advanced Therapeutic Medicinal Product).

1.1.2 Manufacturing Challenges in Cell Therapy

The key raw material for cell therapy manufacture is a cell type obtained from a human source. A key distinction between cell therapies is whether the cell type or its differentiated or otherwise modified derivatives are destined for a single patient or for many patients. A second difference is whether the cells of origin are administered to the patient from whom they are taken. If they are, then they have the genetic identity of the patient and the therapy is autologous. If they are not, then the cells are genetically distinct from the patient recipient and the therapy is allogeneic. For the most part, allogeneic therapies are one to many, while autologous therapies are one to one, though there are examples of one to one allogeneic therapies in which a single patient is treated with cells from a single genetically distinct donor (see Box 1.1).

The manufacture of one to many cell therapies closely resembles the manufacture of biopharmaceuticals (Figure 1.1). Cells from the donor are grown, separated and characterized to make a master cell bank (Box 1.3). Working cell banks can be derived from the master cell bank and used to manufacture patient doses, as for biopharmaceuticals. However, there are important and challenging differences (see Figure 1.1).

We have already briefly mentioned one difference. It is the cells themselves, not their products that are the therapy; as is often remarked, in biopharmaceutical manufacture, one throws away the cells, while in cell therapy manufacture, one throws away the medium. A second crucial difference is that biopharmaceutical manufacture relies on a few standard cell types in standard media; cell therapy manufacture is bespoke to each therapy and does not have the benefit of well-developed platform technologies, for the time being at least.

One to one cell therapy manufacture uses many of the underlying processes and principles of biopharmaceutical manufacture, but is markedly different in scale and separation technologies. Each dose is manufactured for a single patient from a single donor and multiple doses must be manufactured in parallel (Box 1.4).

1.2 The Cell Therapy Landscape

The REMEDiE project has identified around 700 companies that are working in regenerative medicine products or services worldwide, with the large majority located in the US or Europe. More than 90%

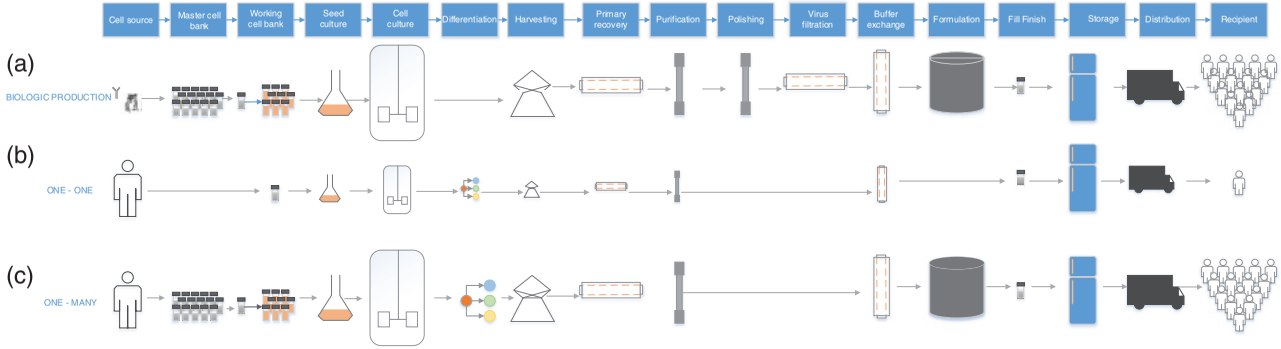

Figure 1.1 Process flow diagram of the sequence of unit operations used in biopharmaceutical manufacture and their relative scales: (a) general process used for the manufacture of monoclonal antibodies; (b) manufacturing for a one on one therapy; and (c) manufacturing process for a one to many cell therapy process.

Box 1.3 How many cells? The scale of manufacture

Mason and Dunhill (2009) and Simaria (2014) have provided some estimates of the numbers of cells that may need to be manufactured. Individual cell doses range from 10^5–10^8. A one to one therapy such as cartilage repair needs a dose of around 10^7 cells and can scale to around 10^4 patients. Treatment for heart failure (one to many) may require a 10^9 dose in 10^7 patients, that is, the manufacture of 10^{16} cells. If cells weigh around 10^{-8} kg, this is around 100,000 metric tonnes: a manufacturing challenge.

Box 1.4 Major manufacturing challenges

Bespoke laboratory based manufacturing processes for early clinical trials may not scale economically to satisfy demand related to disease prevalence. Thus there is a need for early thinking in process development:

- developing robust, replicable processes that can be scaled up or out
- moving early lab-based processes to GMP-compliant processes, materials and equipment; and
- incorporating supply chain and clinical delivery in process development.

Funding process development is a challenge given current public and private funding frameworks.

of these companies are SMEs. Regenerative medicine is defined as including cell therapies, but not exclusively, so it is likely that the number of companies working specifically on cell therapies is somewhat smaller.

1.2.1 Licensed Cell Therapy Products

There are currently 11 cell therapies licensed by the FDA (2014). Of these, five are cord blood derived haematopoietic progenitor cells (HPCs), three are based on fibroblasts or keratinocytes or both, two are chondrocyte-derived and one is a modified dendritic cell. In Europe, only two cell therapies have so far been approved by the EMA and both are chondrocyte-derived (Tozer, 2011). The HPCs are

allogeneic one to one therapies for bone marrow disorders delivered by clinicians; of the three skin cell therapies, two are allogeneic and all are marketed by companies; the chondrocyte therapies are autologous and also marketed by companies. Licensed therapies in Europe and the US are described in Figure 1.2.

1.2.2 Companies, Clinicians, Products and Procedures

In analyzing the cell therapy landscape we have found it useful to distinguish between a cell therapy that can be readily administered to a patient and another that requires a more complex clinical intervention to deliver it to the site of choice. The former we have called a product because it is closest to an off-the-shelf drug or biopharmaceutical. We have classified the latter therapies as requiring a procedure (Foley and Whitaker, 2012). From clinical trial data, the tendency is seen to be for procedure-based therapies to be set up and delivered by clinicians, while product-focused cell therapies tend to be developed by companies. Mapping of the existing licensed therapies shows that 10 involve a clinical procedure and 3 a product. Companies have developed all the licensed products, while the procedures have been developed equally by clinicians and by companies (Figure 1.2).

1.2.3 Cell Therapy Clinical Trials

In an exhaustive analysis of worldwide clinical trials databases, around 1,000 cell therapy trials were found that were not investigating established cell therapies for an established indication (Li et al., 2014). Of these 1,000 trials, just over 400 studied mesenchymal stem cells and an equal number used haematopoietic stem cells. The number of trials based on other stem cell types was 208, including only 6 that used embryonic stem cells, the remainder being somatic stem cells. Around 600 trials used autologous cells and 300 allogeneic cells as a therapeutic agent; around 100 trials involved stimulating endogenous cells with a non-cellular therapeutic.

These figures are broadly comparable to an analysis undertaken in 2011 (Foley and Whitaker, 2012) that used a sampling algorithm using data only from www.clinicaltrials.gov, which might be expected to show a bias towards US and European cell therapy trials. Using the same methodology, we have now analyzed the trials database again; however, this time including additional trials that were registered up

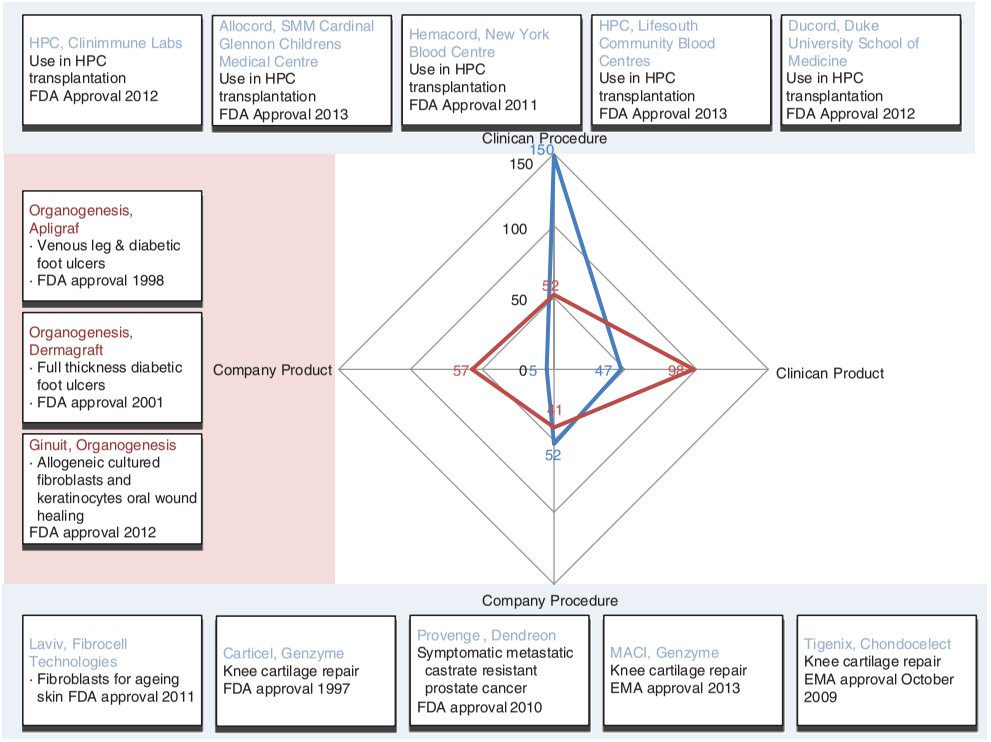

Figure 1.2 Heuristic classification of a selection of cell therapies to the three dimensions. Therapies shown in red are one to many, and therapies in blue are one to one. Numbers are our estimate of all stem cell trials in the clinical trials database that met our criteria. Therapies in boxes have received a market authorization. Abbreviations: EMA, European Medicines Agency; FDA, US Food and Drug Administration; HPC, human progenitor cells.

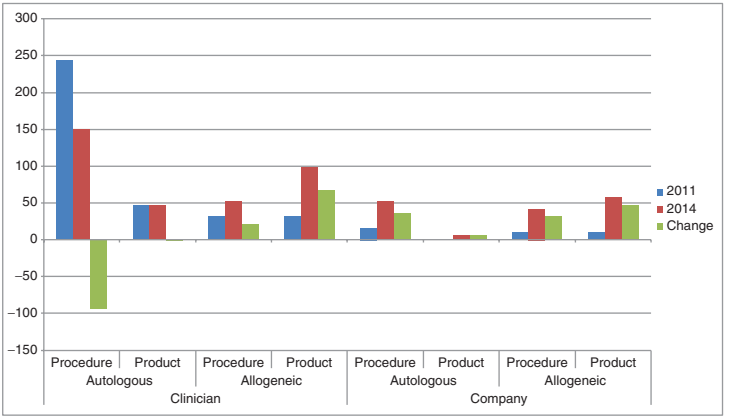

Figure 1.3 A comparison between 2011 and 2014 of the change in cell therapy clinical trials using www.clinicaltrials.gov and the search term "stem cells". Hematopoietic stem cell clinical trials and those terminated before completion were excluded. The trials were analyzed using three dimensions, clinician-company, autologous-allogeneic and procedure-product.

to March 2014 and excluding those that were terminated before completion (Figure 1.3).

There have been some substantial changes in the categories of cell therapeutics and in the relative involvement of companies and clinicians in the three years from March 2011. The number of trials using easily administered products has more than doubled; the number of trials involving procedures remains unchanged, thus products are now represented in around 40% of trials, up from around 17% in 2011. The number of companies sponsoring cell therapy trials has increased four-fold: these company-led trials now account for 30% of trials in the database, up from 10% three years ago. The number of trials of allogeneic therapies has also risen substantially, tripling in the last three years; now half the trials involve allogeneic therapies, up from 20%. Strikingly, the number of trials of clinician-led autologous procedures has fallen by 40% and there have been marked increases in trials of allogeneic therapies sponsored by both clinicians and companies (Figure 1.3).

The analyses of cell therapy clinical trials presented here and previously (Foley and Whitaker, 2012; Li et al, 2014) exclude cellular therapies for cancer. Cellular cancer therapies broadly involve the modification

of immune cells to sensitize them to neoplasms. They are in general one-to-one therapies. The FDA has licensed 11 products to date, including Provenge for prostate cancer (Figure 1.2). Fifteen of the 41 stem cell trials identified in the UK by the Cell Therapy Catapult (an organization fostering and supporting stem cell therapies, including their manufacture) were immune cell therapies, mostly for cancer (Mount, 2014).

1.3 Operations in Cell Therapy Manufacture

The supply chain for cell therapies is complex. It begins with isolation of a cell source from the donor and terminates in delivery of the cell dose to the patient. Processing steps for the two key types of cell therapy, one to one and one to many follow similar paths, though the scale of operation is different (Figure 1.1).

Once the cells have been sourced from a donor or working cell bank, the manufacturing processes for cell therapies involve a number of unit operations (Figure 1.4). First there are unit operations to multiply the number of cells to obtain the quantity required for therapeutic dose(s) and perhaps to differentiate them; these unit operations are described as upstream processing. Secondly, the cells are purified out of the culture media used to grow them so that they are suitable for administration to a patient; the unit operations employed to do this are described as downstream processing. Prior to shipment, cell therapies must be formulated and packaged into a therapeutic dose, this being described as formulation, fill and finish. Finally, at the site where the cell therapy is to be administered, there will be some reformulation and preparation. The next section will outline the unit operations used in the processing of cell therapies from the cell source through to delivery to the patient.

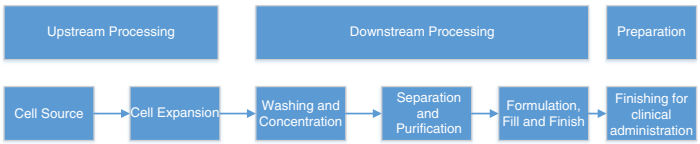

Figure 1.4 Operations performed in upstream and downstream processing of cell therapies.

1.3.1 Cells for Cell Therapy Production

1.3.1.1 Cell Source

Initial cell samples are retrieved from a human volunteer or patient. The tissue source of cells varies. Cell therapies in development illustrate retrieval from a wide variety of sources; biopsies of the skin, hair follicles and eye are common, as are retrievals from peripheral blood and bone marrow (Hipp and Atala, 2008). Procurement regulations and guidelines require ethical approval and patient consent for the biopsy and the uses to which cells are put. In particular, cells that will result in a commercial product require specific consent for this purpose (Box 1.5).

A major difference between one to one and one to many therapies is that cells that are retrieved for one to one therapies are often limited in supply, so a large proportion of the sample will be used as the therapeutic dose. Furthermore, the inward supply chain (shipment of a patients cells or biopsy) for one to one cell therapies is more time critical and requires greater attention to scheduling than one to many cell

Box 1.5 Chondrocelect

Chondrocelect is a Tigenix NV (Leuven) product. It is the first ATMP has been approved by the EMA (Pearce et al., 2014). It is a one to one treatment for cartilage lesions and is manufactured by Pharmacell in the Netherlands. The manufacturing process consists of a biopsy being retrieved from the patient in a hospital setting. The biopsy is then shipped to the Netherlands where the cells are expanded and the final product formulated. This must then be shipped back to the patient where it is implanted into the knee in a hospital. This means two hospital appointments must be scheduled, the timing of the latter dependent on how well the patient's cells grow. Furthermore, product shelf life is a time limiting factor in the shipment. This is further complicated by the release tests the company must conduct prior to the release of the final product, which take time and impact product shelf life. To some extent, the limited shelf life and time delay in receiving release test results has been overcome by introducing a cell preservation step into the process (Vanlauwe et al., 2014). However, the uncertainty around the inbound supply chain, cell growth due to quality and scheduling hospital times still remains.

therapies (Wei Teng et al., 2014). Cells retrieved for one to many cell therapies must be proliferative enough to generate a master cell bank and further working cell banks from which many patients can be treated. As a consequence of these differences, one to one cell therapies usually require little or no long-term storage of either the donated cells or the therapeutic product. For one to many cell therapies, preservation and storage whilst maintaining the ability to proliferate is mandatory. Proliferative capacity in itself is not sufficient: master cell banks and working cell banks must proliferate without phenotypic or genotypic variation and consistently.

1.4 Upstream Processing of Cellular Therapies

1.4.1 Cell Separation

After retrieval of cells from the donor, it is often necessary to separate out the required cells prior to their expansion. Cell labelling and magnetic separation or density gradient separation are usually applied to complete this (Box 1.6). Cell separation may form part of a procedure to purify cells for direct administration to the patient. In this case, the cells are considered to be minimally manipulated.

1.4.2 Cell Expansion

When the retrieved cell sample does not contain enough of the required cells for a therapeutic dose, expansion of the isolated cell population is required. This expansion step means that the cells are no

Box 1.6 Sepax

Biosafe have a centrifugal cell processing system called the Sepax. This system is a fully automated cell separation device that is primarily used for the separation of cord blood, bone marrow and peripheral blood. It is the blood processing industry standard and is GMP, GLP and Point of Care compliant. The Sepax technology is also used in cell separation, washing and concentration of cell therapies and is currently a part of the manufacturing process for cell therapies in clinical trials, for example the Cell Medicas Cytovir CMV product which is in stage 3 clinical trials.

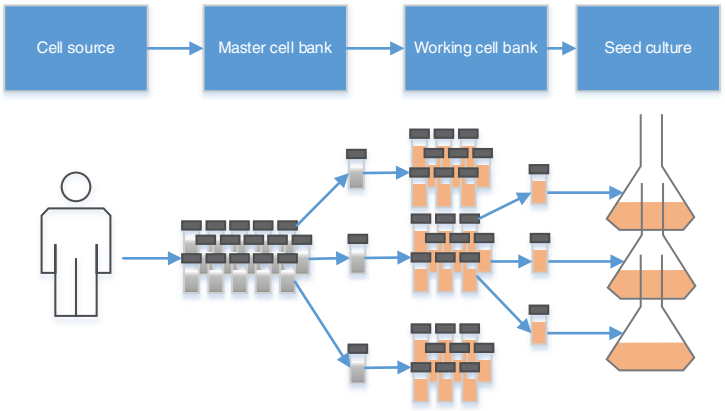

Figure 1.5 Cell banks are created for sustained manufacture of a cell therapy. From a master cell bank, working cell banks are developed which are then used in the manufacturing process.

longer minimally manipulated, so more complex manufacturing regulations apply. In terms of scale, for one to one therapies, the cells must be expanded sufficiently to provide a sufficient therapeutic dose to treat the individual patient. However, for one to many cell therapies, expansion is at multiple levels. Firstly, the cells must be expanded to create a master cell bank that is stored. Each vial from this cell bank then creates a working cell bank from which a single vial can be expanded to create enough cells to treat multiple patients (Figure 1.5). The proliferative capacity of the working cell bank must be sufficient to ensure genomic and phenotypic stability, not only at the point of withdrawal of an aliquot for manufacture, but also during any further proliferative steps during the manufacturing process itself.

We will discuss here three types of cell expansion: tissue expansion, adherent expansion and suspension expansion. Chapter 2 will address the challenges of industrial-scale cell expansion.

In all of the technologies used for cell expansion for cell therapies there has been a ready adoption of disposable materials that come into contact with cells. This limits the risks of contamination but also provides a more cost-effective option when compared with the cost of cleaning and validation of cleaning processes. However, the use of disposables brings the challenge of integrating manufacturing products supplied by different vendors into a seamless manufacturing process.

1.4.3 Tissue Expansion

For skin and for corneal biopsies (as described in Chapter 8) expansion occurs on a flat surface and the tissue sample, given the correct culture environment and media, will expand outwards. This process of expansion is limited by the tissue's ability to expand outwards as it would in its natural environment. This sort of expansion is therefore limited in scalability. Production of cell therapies in this form is thus limited to small surface area one to one treatments. One to many cell products require manufacture at scale and for adherent cell expansion this involves manufacture in parallel (scale-out); ideally, automation is used in order to reduce the otherwise large manpower costs of scale and associated processing risks that arise in manual manufacture at scale.

1.4.4 Adherent Cell Expansion

For most human single-cell expansion to date, the environment found to be most suitable is a flat surface, though this environment does not fully mimic the 3D microenvironment of much of the human body. Adherent cell suspension is the technique in which cells are expanded on a surface, their adherence to which provides stability throughout their expansion. Adherent culture is the most common type of cell expansion for cell therapies and can be commended as a technique that has enabled many cell therapies to enter clinical trials; it is the most widely used (in its simplest form) in discovery laboratories. The simplest type of adherent cell culture is through the use of plastic ware, for example Petri dishes or T-flasks. Cells are grown on the surface of a T-flask in an incubator that controls the environment. When the cells reach a specific cell density they are re-suspended and the flask contents are divided into further flasks for continued growth; this is termed a "passage". There are many advantages to this technique in that it is easy to implement and is well documented. However, the use of T-flasks limits scalability and is usually a manual and open process; thus it is subject to operator variation and the risk of contamination is high unless the processes can be automated.

1.4.4.1 Multi-layer Reactors

The use of T-flasks limits the degree of scalability, since to achieve scale, more and more T-flasks must be used with costs and risks rising rapidly with scale. One approach has been to automate the production

Box 1.7 Bioreactor type comparison

Work done at UCL (Simaria et al., 2014) looks at the number of cells required per dose for one to many cell therapies and the scale of production. A model has been developed that looks at the maximum productivity of different types of reactor used for cell expansion. Based on a patient dose of 10^9 cells the maximum feasibly achievable in T-flasks (80 500 mL flasks) is 0.9 of a dose, in an 80 ten-layer reactor it is 11 and in 8 2,000 L bioreactors using micro-carriers it is 4,708. This technology shift drastically reduces the cost of goods from $49 per million cells in T-flasks and $15 per million cells in multilayer reactors to 70 cents per million cells in bioreactors using microcarrier technologies.

of therapies in T-flasks; systems such as Sartorius Stedims Compact SelecT can process up to 90 T-flasks in one process run. Another approach has been through the development and use of "multi-layer reactors" (as described in Chapter 2). Multi-layer reactors provide the 2D environment required for adherent cells to grow by stacking many surfaces on top of one another within a single container. In essence, they may be thought of as many T-flasks surfaces stacked together in a single vessel. This increases the footprint productivity of a manufacturing facility. Many different types of multi-layer reactor are available, ranging from the most basic to compact versions and automated closed versions that re-circulate and refresh media (Box 1.7).

Multilayer reactors go some way to overcoming the scalability of adherent culture and are adequate for the manufacture of cell therapies where the dose size and target patient population are relatively small.

1.4.4.2 Hollow Fibre Reactors

Hollow fibre reactors are also used for adherent cell culture. For a given volume of container they maximize the surface area required for cells to adhere and grow whilst also maximizing the nutrient and oxygen exchange to the cells. There are many hollow fibre reactors on the market but all follow the same principles. The nature of hollow fibre bioreactors means that they are continuous, closed systems generally supported by a level of automation that facilitates media flow and mass transfer. However, as many of these bioreactors were developed for products that are secreted from cells, process

Box 1.8 Manufacture of Multistem

Multistem is a cell therapy product under development by Athersys. It is an allogeneic therapy which comprises of mesenchymal progenitor cells (MPCs) and is in clinical trials to treat inflammatory and immune, cardiovascular and neurological conditions (www.athersys.com). Early development of Multistem was completed using 2D culture such as T-flasks, and current GMP manufacture for clinical trials is completed in multilayer reactors by their contractor Lonza Biologics, Walkersville. In 2014, Athersys announced a partnership agreement with Terumo to develop their production process for MPCs using Terumo's hollow fibre reactor system, the Quantum. The Quantum is a closed, automated hollow fibre reactor that streamlines the cell culture process and reduces labour costs by up to 80% (www.terumobct.com). It is believed that both manufacturing routes will proceed in order to meet the wide variety of indications intended for this product. However, the Quantum will facilitate a more robust process due its closed, automated design.

optimization has been required to improve the yield of cells: in our hands, high yields are now possible. Whilst hollow fibre reactors are limited in their scalability (Hambor, 2012) they are nonetheless used successfully in a commercial setting for the manufacture of Multistem (Box 1.8) (Chapter 3). However there currently remains a strong desire to develop to suspension cell culture where the productivity per footprint, automation and process control are potentially much greater.

1.4.4.3 Scaffolds

Scaffold technology can also be used to grow cells for therapy. This is more usual for cells that require a 3D structure for administration, for example bone replacement. Scaffolds provide a micro-environment that is favourable for the cells to expand on and provide a structure through which they can proliferate. Cell therapy scaffolds can then be administered directly to the patient to provide a structure that will either remain indefinitely, such as Genzyme's MACI (matrix applied characterized autologous cultured chondrocytes) (De Bie, 2007) or degrade over time, such as the polymer scaffolds developed by Kanczler et al. (2008). There are many types of scaffolds under investigation. These can be from natural sources such as proteins and

polysaccharides or synthetic sources such as polymers, peptides and ceramics (Willerth and Sakiyama-Elbert, 2008). The next generation of surfaces and environments need to be developed to mimic both developmental and disease niches. This should generate cells of enhanced functionality and increase yields.

1.4.5 Suspension Cell Expansion

Suspension cell expansion (as described in Chapters 2 and 4) is the method of choice for the production of biopharmaceutical drugs where the cells are used as factories to produce a therapeutic protein. Suspension cell culture has been developed over many years for the relatively few cell lines used in the manufacture of biopharmaceuticals. Suspension culture maximizes cell yields produced on a small footprint, using cell lines adapted to suspension culture. However, in the production of cell therapies, where the cell itself is the product, the ability of cells derived from normal tissues to proliferate and remain a viable therapy in suspension culture is limited. Thus the optimal conditions for human cells used in cell therapies growing in suspension culture are still very much in development (Kirouac and Zandstra, 2008).

There is, however, a compromise. The bioreactor technology most familiar to the industry can be used in combination with microcarriers to provide cells with an adherent environment to grow on in a suspension culture vessel (explored in more detail in Chapter 4). Microcarriers are small spherical coated or porous beads that provide cells with an environment to adhere to and on which to grow whilst capitalizing on the well-mixed environment of a bioreactor. This well-mixed environment provides optimized exposure to nutrients in the cell culture media and oxygen required for growth and also limits exposure to growth inhibitors produced through cell metabolism. Increasingly, plastic and composites are now being used to push yields higher and aid cell recovery, including the use of thermoresponsive polymers.

1.4.5.1 Stirred Tank Bioreactors

Stirred tank bioreactors are the most common bioreactors used in the biopharmaceutical industry and have been employed with some success in the production expansion of human cells for cell therapies in combination with microcarriers (Kirouac and Zandstra, 2008). Stirred tank bioreactors contain impellers of various designs that gently mix the culture, keeping the cells adhered to microcarriers in suspension,

whilst also allowing nutrient and gas transfer. Without a significant step change in unsupported suspension culture (Kirouac and Zandstra, 2008), stirred tank bioreactors and microcarriers are currently the only plausible hope for mass production of cell therapy products (Simaria et al., 2014).

1.4.5.2 Rocking Platforms

In 1996, Vijay Singh invented the "Wave" bioreactor (Singh, 1999). These rocking platforms possess all of the benefits attributed to stirred tank bioreactors without the need for an impeller, thus reducing shear forces and the risk of mechanical breakdown. The more gentle approach to mixing is often more suited to cells that are sensitive to shear stresses, as human cells are. Though rocking platforms are limited in scale they are often used in the seed train (the pre-culture needed to reach large production scale in stirred suspension bioreactors using microcarriers) and for smaller-scale production for patient-specific products.

1.4.5.3 Perfusion Cell Culture

Perfusion cell culture is fast emerging in cell therapy processing. It allows cells to grow to higher densities in bioreactors by providing fresh medium and removing waste products continuously. Perfusion devices are attached to the bioreactor and provide low shear environments that can be used for media exchange and cell removal in continuous cell culture. Continuous cell culture is the process by which cell removal and media replacement take place continuously to extend the cell culture time and also productivity, that is, the cell number to medium ratio. This method has merits in efficiency and reducing the downtime of equipment and thus increasing the amount of cells manufactured per unit time.

Perfusion cell culture can also be used for washing and concentration of therapeutic cells. Media exchange from one formulation to another can occur in a perfusion reactor and cell concentration is also possible by reducing the ratio of inflow to outflow (Box 1.9). It is a low shear process that reduces the potential for contamination, as the culture broth is not exposed in a separate unit operation.

1.4.6 Differentiation

Differentiation is the process by which pluripotent stem cells are forced down a specific lineage to produce the cell of interest, in this

Box 1.9 Alternative tangential flow

The Alternative Tangential Flow (ATF) system from Refine Technology is a perfusion module that can be added to a stirred tank bioreactor. The module consists of diaphragm, which is pumped up and down, connected to a filter housing and then attached to a traditional bioreactor. Medium exchange can be easily performed using the ATF system, leading to higher cell density batches. The ATF device can be used with both cell and cell-microcarrier systems. When the ATF device is used with a system that uses microcarriers to grow cells, it is possible to perform further process steps in a single bioreactor (e.g. enzymatic detachment of cells from microcarrier beads) and thus reduce downstream processing. These steps are concentration, wash, perfusion, rapid medium exchange, trypsinization and cell-microcarrier separation (www.refinetech.com).

case the therapeutic cellular therapy product. Often the cell of interest would not proliferate or would do so very slowly and so the precursor cell to the type required is cultured to the quantity desired and then differentiated into the cell type of interest. Differentiation is a process step that is carried out by making additions to the culture media that force the pluripotent or multipotent stem cells to turn into the required cell product. Beyond this step, in the downstream processing, care must be taken to remove any undifferentiated cells from the therapeutic dose. Pagliuca et al. (2014) have demonstrated that differentiation is possible at scale. They were able to differentiate, in suspension, at a 500 mL scale, human pluripotent stem cells into functional pancreatic beta cells that comprised around 10% of the final cell population.

1.5 Downstream Processing of Cellular Therapies

1.5.1 Harvest, Washing and Concentration

After cell expansion, the next steps in cell therapy processing are to wash cell culture media from the cells, re-suspend in a non-proliferative media and concentrate into a volume more suited to further processing. The techniques traditionally used derive from the blood

processing industry, but there is a desire to move towards the more scalable methods of traditional biopharmaceutical processing, particularly for allogeneic cell therapies. These techniques are discussed in detail in Chapter 5.

1.5.1.1 Centrifugation

Centrifugation is a unit operation that is traditionally used to separate cells from the supernatant and concentrate cells after cell culture. Centrifugation is used in traditional biopharmaceutical manufacture where the cells are no longer required and thus the processing step is designed to collect the protein present in the supernatant. Traditionally then, this step is used to remove the cells as opposed to preserving them.

Batch centrifugation is the most commonly used laboratory method. Cells can be separated from the supernatant using centrifugal forces in a batch approach. This has some shortcomings when used for cell therapy manufacture. Firstly, the removal of cells from the centrifuge bowl can case cell damage and, secondly, the batch centrifugation process is limited by scale.

Continuous centrifugation (described in Chapter 5) has been used with success in the processing of cell therapies. Orthogonal continuous centrifugation, where fluid flow is perpendicular to the centrifugal force, can be used to concentrate cells but is limited in its washing capability. Counterflow centrifugation, where fluid flows in the opposite direction to the centrifugal force, allows both cell washing and concentration. Counterflow continuous centrifugation has been developed and used with success in cell therapy manufacture; its scalable nature and use of a single-use fluid path provides a suitable solution for the gentle washing and concentration of human cells (Pattasseril et al., 2013).

1.5.1.2 Filtration

The use of normal flow filtration (where pressure and fluid flow is perpendicular to the filter) and tangential flow filtration (where fluid flow is along and parallel to the filter and a perpendicular pressure is applied) is routine in traditional biopharmaceutical processing. However, normal flow filtration is generally used for sterilization and does not handle large volumes of liquid well. Tangential flow filtration is used for separation processes in biopharmaceutical manufacture, but it employs high trans-membrane pressure that is not suitable for cell therapies, as it would compromise cell viability due to high shear

forces. Industry has now developed tangential flow filtration devices with low shear effects through the application of low transmembrane pressures that are suitable for use in both concentrating and washing human cells (Pattasseril et al., 2013).

Filtration devices range from the most basic to fully automated and as they are mostly disposable they will integrate well into a cell therapy manufacturing process. The capital set-up cost for filtration devices is significantly less than for centrifugation. In addition, the fluid path remains enclosed, so risk of process failure is lower, and environmental background requirements are also lower than for centrifuges. Such potential advantages may lead to filtration devices becoming more prominent in cell therapy processing (Pattasseril et al., 2013).

1.5.2 Separation and Purification

After cell concentration and media exchange, the final steps in cell therapy processing are separation of the desired cells and purification to remove any unwanted residuals in the solution. For traditional biopharmaceuticals, a combination of filtration and chromatography steps are used. For cell therapies, density gradient centrifugation and/or magnetic separation are the preferred methods (Pattasseril et al., 2013). Though an outline is provided on current cell separation technologies here, this topic is covered in more depth in Chapter 5, where novel methods will also be discussed.

1.5.2.1 Centrifugation

Centrifugation is used to purify cellular products as well as in cell recovery. Centrifugation separates the bulk mixture based on its composition in terms of density and size. This process step is designed so that the fraction that contains the cells of choice is harvested for further processing. Density gradient separation has been made more accurate through operation in counter flow mode. Centrifuges have been used to process blood for some time, an example of this being the Terumo BCT's Cobe 2991 system that performs blood apheresis using continuous centrifugal technologies. The scale of this system is limited to less than 1 litre and it is best used for one to one cell therapies. In traditional biopharmaceutical production, centrifuges are widely used. However, they are focused on the removal of cells from the supernatant that contains the product. As the cells are a by-product in traditional biopharmaceutical manufacture there is little requirement

to keep the cells intact, with the caveat that cell disruption can make subsequent downstream processing steps more difficult. The kSep from KBI is a low shear centrifuge system that has shown great promise in cell therapy processing Box 1.10). It is a continuous centrifuge with a disposable flow path that achieves 80% cell recovery whilst maintaining greater than 90% cell viability (Pattasseril et al., 2013) and is able to process 720 L/h (www.ksepsystems.com).

Box 1.10 Approaching the problem from two directions

The first cell therapies to be adopted used bone marrow stem cells for which a manufacturing approach is well established. In the UK, this manufacturing is carried out in hospitals and each batch is processed separately. Though processing blood for blood transfusion is centralized, each unit of blood that is donated is also processed separately. This means a raft of technologies have been developed to service blood and bone marrow processing, which is effectively a one to one therapy. These technologies have made their way into cell therapy processing due largely to hospitals and universities being the prime location for early stage cell therapy development (Brandenberger et al., 2011). Centrifugal techniques, such as Terumo BCTs Cobe 2991 (www.terumobct.com), have been used in cell therapy processing. The scale for which these technologies were developed however, means their application in cell therapy processing at scale is limited.

The biopharmaceutical industry is used to manufacture large batches of therapeutic proteins and technologies have been developed to complete the steps required for these one to many therapies. Though not entirely suited to the need to retain the cells as the therapeutic product, these unit operations and processes have been developed and used by industry to produce large batches under comparatively strict manufacturing regulations to those for ATMPs. Centrifugal technologies such as Pnuematics Unifuge (www.psangelus.com) and KBIs kSep (www.kbibiopharma.com) have been developed using the principles of the biopharmaceutical industry, whilst also dealing with the sensitivities around the cells being the final product.

Cell therapy bioprocessing is dealing with both of these challenges, as some products are one to one and some are one to many, depending on the therapy in question; however, all must comply with the regulations of drug manufacture.

1.5.2.2 Magnetic Separation

Magnetic separation is analogous to affinity chromatography steps in traditional biopharmaceutical manufacture in that it uses a property of the surface of the cell required to perform a highly accurate separation (Box 1.11). Identification of a surface antigen unique to the cell of choice is required. An antibody specific to this antigen is attached to a magnetic particle that binds to the cells of choice when they are exposed to each other. Thus the cells of choice are retained by a magnetic field and all waste product passes through the column.

1.6 Formulation, Fill and Finish of Cellular Therapies

Unlike traditional biopharmaceuticals, which are usually packaged for delivery to the patient as either a vial or pre-filled syringe for percutaneous injection or as a sterile bag for infusion, cell therapies are administered to patients in many different ways; they may be

Box 1.11 CliniMACs

Miltenyi Biotech was founded in 1989 by Stefan Miltenyi to commercialize the Magnetic-Activated Cell Sorting (MACS) technology he had invented (www.miltenyibiotech.com). The technology involves coupling a magnetic bead to a cell of interest using an antibody specific to that cell. They are then separated from other cells using a magnetic field. The CliniMacs system which automates this process was developed for cell selection processes, but has also found a market for cell separation in bioprocessing of cellular therapies. It has been approved by the FDA for CD34 selection in the treatment of acute myeloid leukemia. The MACs technology has been widely adopted by scientists and has been the subject of over 20,000 publications and has been used in over 40,000 clinical treatments. The CliniMACs has now been further developed and the CliniMACs Prodigy was released in 2013. The CliniMACs Prodigy is a GMP compliant cell processing device that completes both upstream and downstream processing steps in a closed, automated system. The culture chamber of this system only holds up to 400 mL and thus this system is more suitable for one to one therapies.

administered as a sheet of cells, an injection, intravenously or in a more complex operative procedure. This means the final form in which they are delivered to the clinic differs from one therapy to another (see Chapters 6 and 7). However, they all share similarities in the complexity of their administration. Formulation, Fill and Finish is an often neglected area of cell therapy development. It is key, however, to ensuring an efficacious product is delivered. Poor stability can result in business models that are not cost-effective, negating process efficiencies achieved earlier in the process. To ensure robust and reliable delivery of cells, an End to End Supply Chain mentality needs to be utilized, as exemplified by the Seamless Freight activities at the Cell and Gene Therapy Catapult (www.ct.catapult.org.uk).

1.6.1 Formulation

As a final step in processing, the cells should be formulated into a buffer that is suitable for storage. The most effective approach is to ensure that the buffer solution the cells are formulated into may also be suited to administration to humans; this approach is becoming more prominent in the industry. Where this cannot be the case, an additional step must be added in the clinic where a buffer exchange will be required.

1.6.2 Fill and Finish

The cell solution should now be added to the final storage and administration vessel. This could be a vial, a bag, a prefilled syringe or some novel storage device. Packaging live cells has challenges in terms of shelf life and the fragility of cells. The latter challenge requires that care be taken not to damage the cells through shear stresses on the final fill step in the cell suspension.

Many cell therapies are delivered to the patient in blood bags at low cell concentrations that are suitable for small lot sizes, but as lot size increases the use of vials and filling machines at much higher cell concentrations will be necessary (Brandenberger et al., 2011).

There is an increasing need to ensure process data is generated on hold times and temperatures, pre-fill mixing shear rates and fill speed shear, as these will have an impact on stability.

Shelf life of the cell suspension at this stage in the process will often be limited and thus this part of the supply chain must take place as quickly as possible. The volume and number of doses being processed

will have an influence on what is feasible in terms of packaging times, but a general rule is "as fast as possible". There are no intermediate products that can be stored and this limits downstream processing choices and increases the need for real time fill. For some allogeneic cell therapies, large, high throughput filling machines whose overall capacity far exceeds the batch size are required in order to minimize the time for this operation. By way of an example, Provenge, which is a one to one cell therapy, has a shelf life of only 18 hours once packaged and 3 hours once removed from its insulated packaging. By comparison, if the same therapy was one to many and a batch were produced at scale in the bioreactor systems described previously (Simaria et al., 2014), then 4,708 vials would need to be filled; this process could take almost 8 hours using a system such as the Aseptic Technologies LX1 filling line (www.aseptictech.com), which can fill 600 vials per hour. This would significantly reduce the available shipping time for the product. However, a line such as Boschs FLC3000 (www.boschpackaging.com), which can fill 36,000 vials per hour, would take approximately 8 minutes and would have little effect on the available shipping time.

1.6.3 Preservation and Shipment

Over time the development of methods of preservation such as lyophilization have been key to the supply chain of traditional biopharmaceuticals. However, for cell therapies, successful preservation strategies are still very much in development. Cell storage solutions are covered in detail in Chapters 6 and 7.

For cell therapies whose product shelf life cannot be increased through preservation, the outbound supply chain is critical, as the therapeutic product must reach the patient as soon as possible. This is more likely to be the case for an autologous therapy in many instances (e.g. Provenge, discussed above); for an allogeneic cell therapy, it does not make commercial sense to develop a product that cannot be preserved. There are, however, cases where allogeneic cell therapies have minimal preservation. For example, Apligraf in its final form has a shelf life of 5 days (Czaja et al., 2006) and thus significant amounts of product are often disposed of, because they have exceeded their expiry date, increasing overall cost of goods.

Shipment of cell therapies that are not preserved is usually carried out at low temperature to delay metabolism and cell death (Chapter 7).

Transportation methods exist for biopharmaceutical therapies, blood and organs. However, good distribution practice dictates that the mode of transport must ensure that the product has not been exposed to elements that may compromise their quality and integrity (Coopman and Medcalf, 2014). Temperature monitoring and control has historically been the biggest challenge; however, Nikolaev et al. (2012) also demonstrated the negative effects of vibration on mesenchymal stem cells stored at 4°C.

Some cell therapies can be cryopreserved (see Chapter 6). That is, they are mixed with a buffer that protects the cells and can be stored at ultra low temperatures (–178°C). Controlled rate freezers are used to gradually cool the cells to this temperature. Traditionally this is achieved through the use of liquid nitrogen. More recently, novel approaches have reached the market, such as Asymptotes (www.asymtote.co.uk) VIAFreeze technology that is based on Stirling engine cooling. Once cryopreserved, cells for therapeutic use can be stored for significant periods but the outbound supply chain should be considered; for example, local cryogenic storage temperatures may be higher than in the manufacturing facility. If reformulation is not carried out in the clinic once the therapeutic dose comes out of cryopreservation, this must be done prior to shipping. Shipment periods should then be kept to a minimum.

Preservation of cell therapies not only reduces the risk in the outbound supply chain but also allows time for quality control test results to be returned and the product released by a Qualified Person with a complete dataset and reduced risk. Very short shelf lives mean that some key quality control data are often returned after release and administration to patients; this is less than ideal.

1.7 Administration of the Cell Therapy to a Patient

The final steps required to deliver a cell therapy to the patient have the potential to be the weakest link in the supply chain. A major challenge is consistency between sites during the process of preparing the cell therapy for administration to the patient. Ideally there should be no reformulation at the bedside. Even without reformulation there is a possibility of variability, for example, cryopreserved products

require thawing and this may not be carried out reproducibly from one clinical site to another. However, automation to control and record cell thaw and low-risk preparation steps are being developed by companies in the field. A long-term aim for this final step in the supply chain must be automation to reduce variability and human error to a minimum.

1.8 Cell Therapy Manufacturing Facilities of the Future

Given the history of the emergence of the cell therapy industry, existing manufacturing facilities are largely designed to accommodate processes that are open and compromised by non-sterility. This means that the majority of manufacturing suites are small, high-grade clean rooms where processes are manually undertaken under controlled conditions. This route to manufacture is more suited to one to one therapies, where the production of each dose is a separate process and therefore should be self-contained. When considering this process route for one to many therapies, its labour intensity has to be markedly amplified to achieve the volumes of doses needed.

The ability to produce cell therapies on an individual or small batch basis in these high-grade clean rooms has been the basis of manufacture to date and has served the needs of early phase clinical trials adequately. However, as the industry matures, as there are more cell therapies in development and as a greater number reach later stage clinical trials, so there is a need to address the current *status quo* for manufacture.

Current processes are, in the main, open. That is, as the process medium moves from one unit operation to the next, it is exposed to the surrounding environment and possible contamination. In order to satisfy regulatory requirements, there is a clear need for high-grade clean rooms and highly trained personnel. If cell therapies are to be manufactured at scale there is, however, a pressing need to move away from a processing route that consists of these elements (high-grade clean environments, open processes and highly trained personnel) to a manufacturing route that reduces both risk and cost of goods, yet still produces therapies that are of the highest quality through closed processes and automation.

In order to move to this next stage, there are some key challenges. The first is that the production methods and scale of production for cell therapies varies widely, from one to one to one to many, from adherent to suspension or scaffolds, from orphan diseases to highly prevalent diseases and so on. The variety of cell therapy products means that a single, standard platform process is not feasible. However, it is possible to foresee a future where a suite of standardized platforms may be available or rather that a range of unit operations available at different scales may be plugged together to form a whole process. The second challenge is the ability to move towards closed processing. If it is possible to close a process so that it is not exposed to the external environment then lower-grade clean room suites can be used. This would then reduce the current high costs of such an environment and would also allow multiple processes to take place at the same time, in the same suite. However, this is challenging for a number of reasons (see Box 1.12).

There is also the challenge of scale. For large-scale production of one to one therapies, the required step is to scale-out the process (i.e. parallel execution of the same process at the same scale occurring at the same time). For one to many cell therapies, there is a need to

Box 1.12 Challenges in moving to closed processing

The current labour intensity of existing processes means that they are subject not only to high specialized labour costs but also subject to risk through operator intervention and variation. Removing the labour intensity of processes via automation and the development of new process technology will help mitigate this cost and risk:

- when dealing with a range of unit operation technologies, as each equipment manufacturer has its own unique inlet and outlet configurations. Fluid paths need to be compiled and shown to be validated.
- There are a lack of industry standards to enable connectivity; both physical with regards to tube interfaces, but also equipment connectivity with regards to data integrity.
- Introduction of a process changes may lead to the need to complete product comparability studies to demonstrate no adverse affect on product.

scale-up the process to achieve production at large scales to satisfy the market demand.

As previously mentioned, manufacturing processes for cell therapy have often been born from blood processing suites in hospitals where the procedure happens close to the patient. This route of manufacture limits the supply chain demands, but will not be suitable or possible for all cell therapies. For one to many cell therapies, it is essential from a cost of goods perspective to follow the processing route of traditional biologics that are manufactured at a central site and then shipped to the patient. This is limited somewhat by the current shelf life of cell therapies, though investment is being made in developing longer shelf life and more stable products (see Chapter 6). It is doubtful that one supply chain will be developed for all cell therapies due to the variation in cell source, disease prevalence and the product's shelf life; thus various solutions have been proposed such as distributed manufacture (local, smaller, manufacturing facilities) or wholly closed automated processes that manufacture what is needed for each patient at the bedside or in the clinic. The latter approach challenges the current structure of the drug manufacturing industry, where each facility that manufactures any sort of drug must be licensed by the regulatory body and each batch of therapy must be released by a qualified person. One approach would be to use remote monitoring by qualified persons and thus real time release. Perhaps in the future the manufacturing devices that sit in the clinic may be classed and regulated as medical devices.

In order to achieve wholly closed automated processes, investment is needed in scale-down, automation, non-invasive sensing, data integration and management Box 1.13). This must be underpinned by

Box 1.13 Invetech

The concept of wholly closed and automated processes has been addressed by Invetech (www.invetech.au). Invetech have developed a closed processing platform for Argos therapeutic cancer immunotherapy which can be operated in a lower-grade clean environment. The process is wholly automated and requires only a small tumour sample from the patient. The therapy is now in Phase 3 clinical trials and demonstrates that early investment in process development and plans for routine manufacture has led to a suitable scalable solution that could indeed operate in the clinic or close to the patient.

predictive manufacturing so that quality is ensured throughout the process and not tested in and at the end by quality control requiring sign off by a qualified person, as is the current model.

1.8.1 Factory of the Future Requirements

Facilities of the future must be adaptable to markedly different product types and scales. Process development must be focused on efficient downstream processes as well as optimizing upstream growth, seamless fill finish, chill, freeze, and possible inactivation. They must also deliver frozen or chilled dispatch and straightforward logistics. The advantages are cheaper air handling equipment that need deliver only a lower level of air grade and much more reliable replication of manufacture, allowing focused delivery of product and the benefit of derisked future process expansion.

As the industry is still young and there are as yet very few cell therapies on the market, it is impossible to say which manufacturing routes will become dominant. However, there is a commonly held view in the industry that open, labour-intensive processing in expensive clean rooms will not adequately service the industry, as more and more cell therapies come through the development pipeline and reach the marketplace.

1.9 Conclusion

The curative potential of cell therapies is showing great promise and thus the cell therapy industry continues to grow as new products emerge on the market and more products enter late phase clinical trials.

The challenges presented by one to one and one to many cell therapies are different; they require different supply chain approaches while adhering to the same manufacturing regulations. Though there are parallels to be drawn with the manufacture of blood products and traditional biopharmaceuticals, cell therapies are the most complex products to manufacture, formulate and administer in the history of therapeutics manufacturing.

In order for the cell therapy industry to realize its ambitions of providing curative therapies, the industry must address the pressing challenges of closed processing through interoperability of machines that carry out unit operations and develop robust manufacture at scale to ensure therapies are cost-effective and widely available.

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